

TECHNICAL ARTICLES

SOLID-STATE FOURIER TRANSFORM INFRARED AND ^{31}P NUCLEAR MAGNETIC RESONANCE SPECTRAL FEATURES OF PHOSPHATE COMPOUNDS

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Solid-state spectroscopic techniques, including Fourier transform infrared (FT-IR) and solid-state ^{31}P magic angle spinning nuclear magnetic resonance (MAS NMR) spectroscopies, are powerful tools for evaluating metal speciation and transformation mechanisms of P compounds in soil, animal manure, and other environment samples. These spectroscopic studies generally rely on the reference spectra of model compounds. However, such reference information is limited, especially for metal phytate compounds, which are an important component of many samples (e.g., 60–80% of soil organic P). In this study, we used solid-state FT-IR and ^{31}P MAS NMR to examine metal phytates and their orthophosphate counterparts, as well as several pyrophosphates and polyphosphates. Comparisons of the FT-IR spectra of metal orthophosphates and phytates demonstrated distinct characteristics and differences. The triplet-peak feature in the 790- to 900- cm^{-1} range could be used to distinguish metal phytates from other P compounds that contained one or no peak in the same range. FT-IR spectral features centered at 1100 cm^{-1} (i.e., broad or splitting multiple peaks) could distinguish among different metal phytate compounds. We observed that the solid-state ^{31}P NMR peaks of phytate compounds were broad, whereas those of inorganic P compounds were much sharper. We propose that the same attention should be paid to spinning sidebands as on the main peaks, because the features of spinning sidebands were unique for some P compounds. Finally, our observations indicated that wetting samples could change both intensity and position of chemical shifts and spinning sidebands, thus keeping samples dry is necessary to obtain repeatable and high-quality solid-state ^{31}P MAS NMR spectra. The solid-state FT-IR and ^{31}P NMR reference spectra established in this work may help in basic and applied environmental soil P studies by the two advanced spectroscopic technologies. (Soil Science 2007;172:501–515)

Key words: FT-IR, NMR, phosphate, phytate, solid-state spectroscopy.

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PHOSPHORUS (P) is an essential nutrient for growth. Thus, in agricultural systems, P is added to cropland as fertilizers or animal manures to maintain P availability and crop production. Continued agricultural expansion during the next 50 years is predicted to be accompanied by a 2.4- to 2.7-fold increase in eutrophication of freshwater and near-shore marine ecosystems (Tilman et al., 2001). Computer models have predicted up to 1000 years or more are required to recover from eutrophication caused by agricultural overenrichment of soils (Carpenter, 2005); although other estimates are much shorter. The causes of this slow recovery, or nonrecovery, are many and not well understood. Improving our knowledge of soil and sediment phosphorous dynamics would enable quicker development of novel technologies and management practices for reducing eutrophication.

Phosphorus is present in the environment in various forms, which differ widely in their stability and bioavailability. Traditionally, the composition and behavior of P in environmental samples have been investigated by solution-based techniques. These methods include sequential fractionation (Hedley et al., 1982; Leinweber et al., 1997; Dou et al., 2002; He et al., 2003; Wienhold and Miller, 2004), enzymatic hydrolysis (Herbes et al., 1975; Toor et al., 2003; Turner et al., 2002a; Dao, 2004; He et al., 2004; He et al., 2006a,b), solubility equilibria (McDowell and Sharpley, 2003), and solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy (Leinweber et al., 1997; Cade-Menun et al., 2002; Koopmans et al., 2003; Toor et al., 2003; Turner et al., 2003). Recently, solid-state spectroscopic techniques have emerged as powerful analytical tools for shedding light on the reaction mechanism of P transformations (Hinedi et al., 1989; McDowell et al., 2002; Peak et al., 2002; Toor et al., 2006). The three major molecular-scale spectroscopic techniques used in environmental P chemistry are Fourier transform infrared (FT-IR), solid-state ^{31}P NMR (e.g., magic angle spinning [MAS]), and X-ray absorption near-edge structure (XANES) spectroscopies. Infrared spectroscopy has been used extensively in characterization and structural studies of phosphate species (Chapman and Thirlwell, 1964; Tejedor-Tejedor and Anderson, 1990; Rulmont et al., 1991; Celi et al., 1999; Arai and Sparks, 2001). Solid-state ^{31}P NMR analysis has been used to assess metal-inorganic phosphate species in soils, wastewater sludges, and animal manures (Hinedi et al.,

1989; Frossard et al., 1994; Shand et al., 1999; McDowell et al., 2002; McDowell et al., 2003; Hunger et al., 2004; Jayasundera et al., 2005; McBeath et al., 2006) and identify calcium phosphate, aluminum phosphate, and pyrophosphate. Investigation of the effects of many monocation, dication, and trication on P K-edge XANES spectra indicates that chemical differences beyond the phosphate tetrahedron effect spectra (Franke and Hormes, 1995; Okude et al., 1999). Thus, P K-edge XANES analysis has been performed to study adsorbed and mineral forms of phosphates (Hersterberg et al., 1999; Peak et al., 2002; Beauchemin et al., 2003; Khare et al., 2004; Sato et al., 2005; Toor et al., 2005).

Much of the effort has been focused on the inorganic P speciation (i.e., metal-orthophosphate interactions), whereas organic P plays an important, if not equal, role in P-recycling in nature (Toor et al., 2006). Phytate (inositol hexaphosphoric acid salt) has been found to be a major component of organic P in animal manure, soil, and other organic substances (Cosgrove, 1962; Herbes et al., 1975; Turner et al., 2002b; He et al., 2006a). About 51 million Mg of phytate is formed in crops and fruits each year globally (Lott et al., 2000). Thus, phytate has often been the focus of study by solid-state spectroscopic technology (Celi et al., 1999; Shand et al., 1999; Peak et al., 2002; Beauchemin et al., 2003; Hunger et al., 2004; Jayasundera et al., 2005; Toor et al., 2005). However, these studies generally have used one or two phytic compounds (phytic acid, Na or Ca salt) to obtain a solid-state phytate spectra (Table 1), leading to the conclusion that their spectra are unremarkable: broad peaks without distinguishing features (Peak et al., 2002; Beauchemin et al., 2003). Phytate, a six-C ring P compound, contains six phosphate groups attached via an ester linkage. Each of the phosphate moieties can interact with various metal ions to form various soluble or insoluble compounds (phytate salts) (Celi et al., 2001; Dao, 2003; He et al., 2006a). He et al. (2006c, 2007) prepared several metal phytate compounds and demonstrated significant differences in the shape and position of spectra among metal phytates (Table 1). Their work indicated that solid-state spectroscopy could be used to advance our knowledge of the fate of phytate, particularly when phytate interacts with metal species in animal manure, soil, and other environment. It is obvious that a wider range of reference spectra of various metal phytate compounds is

TABLE 1
Fourier transform infrared and solid-state ^{31}P NMR spectral data of phytate used in soil P chemistry studies

Spectra analyzed	Instrument	Research type and purpose	Reference
$\text{Na}_{12}\text{IP}_6$	NMR	Applied, P species in soil ingredients	Shand et al., 1999
$\text{KH}_{11}\text{IP}_6$	FT-IR	Basic, absorption behavior	Celi et al., 1999
IP_6 -goethite			
IP_6 -illite			
Na-IP_6^\dagger	NMR	Applied, P species in poultry litter	Hunger et al., 2004
Ca-IP_6^\dagger			
Na-IP_6^\dagger	NMR	Applied, P species in dairy manure	Jayasundera et al., 2005
$\text{Na}_{12}\text{IP}_6$	FT-IR	Basic, model compounds	He et al., 2006c
Ca_6IP_6			
Mg_6IP_6			
Cu_6IP_6			
Mn_6IP_6			
Al_4IP_6			
Fe_4IP_6			
$\text{Fe}_{4-x}\text{Al}_x\text{IP}_6$			
IP_6	FT-IR	Basic, absorption behavior	Guan et al., 2006
$\text{IP}_6\text{-Al}(\text{OH})_3$			
$\text{Na}_{12}\text{IP}_6$	NMR	Basic, spectral feature of metal phytates	He et al., 2007
$\text{K}_2\text{H}_{10}\text{IP}_6$			
Ca_6IP_6			
Al_4IP_6			

† The ratio of metal to IP_6 was not specified.

required to reach this goal. In this study, we examined solid-state FT-IR and ^{31}P MAS NMR features of more metal phytates and their orthophosphate counterparts. We also analyzed spectra features of several pyrophosphates and polyphosphates that occur naturally in soils or have been added (i.e., via fertilizers; Dick and Tabatabai, 1987; Hossner et al., 2004; McBeath et al., 2006).

MATERIALS AND METHODS

Phosphorus Compounds

Zinc and Cd compounds of phytate were prepared from phytic acid dodecasodium salt ($\text{Na}_{12}\text{IP}_6$) and metal chlorides according to Evans et al. (1983), Evans and Martin (1988), and He et al. (2006c). Briefly, $\text{Na}_{12}\text{IP}_6$ was mixed with 0.2 M HCl to make a final concentration of 15 mM. A sufficient quantity of Zn or Cd chloride was added to each of these solutions so that a metal-to-P ratio of 1:1 was obtained in the reaction mixtures. The solutions were very carefully adjusted to a pH of 6 by slowly adding 1 M NaOH dropwise while stirring in a 1-h time interval. The white precipitates were filtered and washed with three 20-mL portions of boiling deionized water to remove any possible adsorbed or occluded metal ions. These washed precipitates were air-dried at

room temperature to a constant weight and kept in a desiccator at room temperature until use. The metal-to-P molar ratio was 0.83 ± 0.02 (mean \pm standard deviation, $n = 2$) for the synthetic Zn compound and 0.97 ± 0.03 (mean \pm standard deviation, $n = 2$) for the synthetic Cd compound, corresponding to $\text{Zn}_5\text{H}_2\text{IP}_6$ and Cd_6IP_6 , respectively. For comparison, Zn and Cd orthophosphate complexes were prepared under the same conditions with Na_3PO_4 and metal chlorides. The metal-to-P molar ratio was 1.30 ± 0.14 (mean \pm standard deviation, $n = 2$) for Zn-PO_4 and 1.41 ± 0.01 (mean \pm standard deviation, $n = 2$) for Cd-PO_4 . The stoichiometry of metal(II)- PO_4 was less than 1.5, probably because of the formation of some dibasic metal(II)- HPO_4 compounds.

Aqueous (40–50% wt./wt.) phytic acid (H_{12}IP_6) was purchased from Spectrum Laboratory Products, Inc. (New Brunswick, New Jersey). The syrupy, strawlike liquid of pure phytic acid was obtained by freeze-drying and subsequent drying in a room-temperature desiccator. Other P compounds were purchased from Sigma (St. Louis, Missouri) or prepared in-house (He et al., 2006c).

Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectra of solid P compounds were obtained using KBr discs.

Each disc contained about 1 mg of sample and 80 mg of KBr. The spectra were recorded from 450 to 4000 cm^{-1} on a Perkin Elmer Spectrum One FT-IR Spectrophotometer (Perkin Elmer Instruments, Norwalk, Connecticut). For the spectrum of phytic acid, a drop of phytic acid was loaded on a Cleatran ZnS disc (International Crystal Laboratories, Garfield, New Jersey). A

layer of phytic acid with proper thickness was formed by putting another Cleatran ZnS disc over the loaded one, squeezing and turning the two discs. The spectrum was obtained from 720 to 4000 cm^{-1} with a blank Cleatran ZnS disc as background. Each sample was scanned 24 times with a resolution of 2 cm^{-1} . All spectra were normalized.

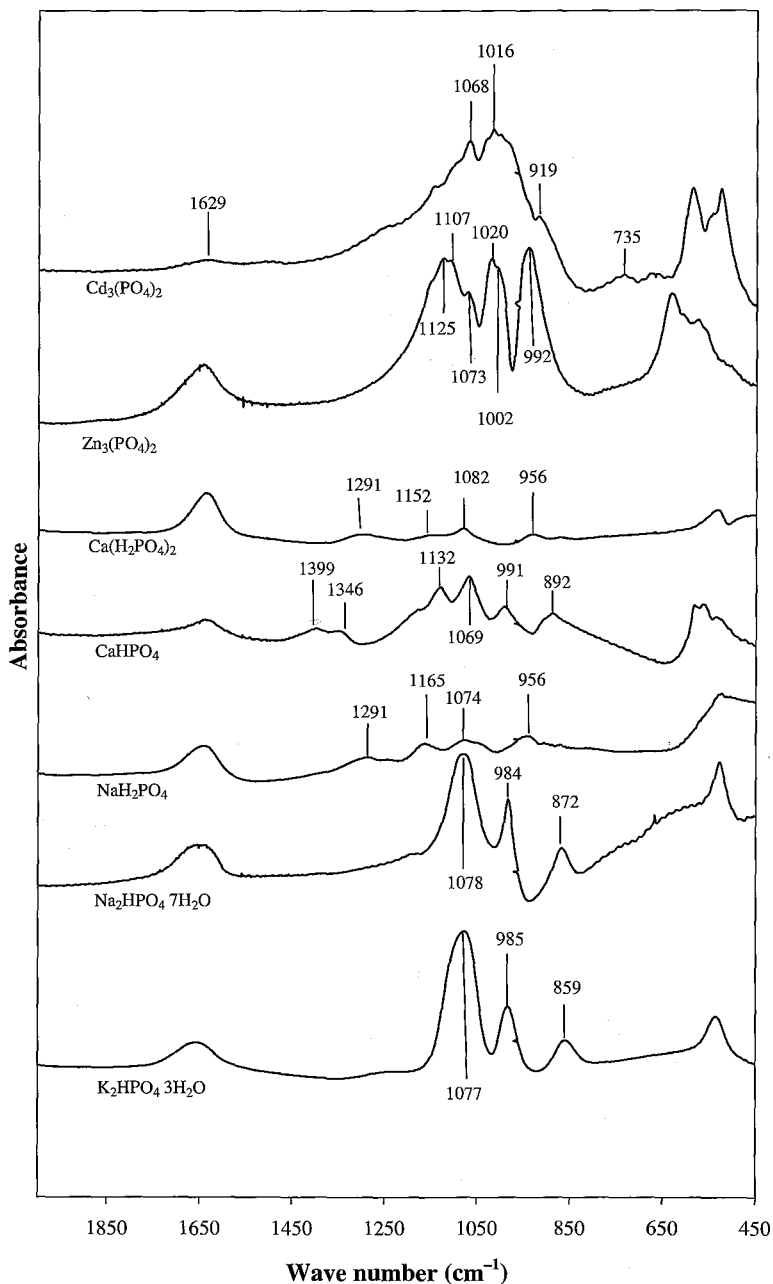


Fig. 1. Fourier transform infrared spectra of metal phosphate compounds.

Solid-State ^{31}P NMR Spectroscopy

Solid-state ^{31}P MAS NMR spectra were collected on a Varian Inova 500 spectrometer operating at 202.489 MHz using a Doty Scientific 4-mm/XC MAS probe. Bloch decays of 50 msec were collected with a 200-ppm window after 30-degree excitation pulses. A relaxation delay of one second was used between each transient. Two-pulse phase modulation

(McGeorge et al., 1999) modulated proton dipolar decoupling with a field strength of 45 kHz was applied during acquisition. A MAS speed of 10 kHz was used, and between 8 to 64 scans were collected for most runs to obtain an sufficient signal to noise ratio of the main peaks.

For comparison purposes, several drops of water were added into NMR tubes of several phytate samples after the initial solid-state MAS

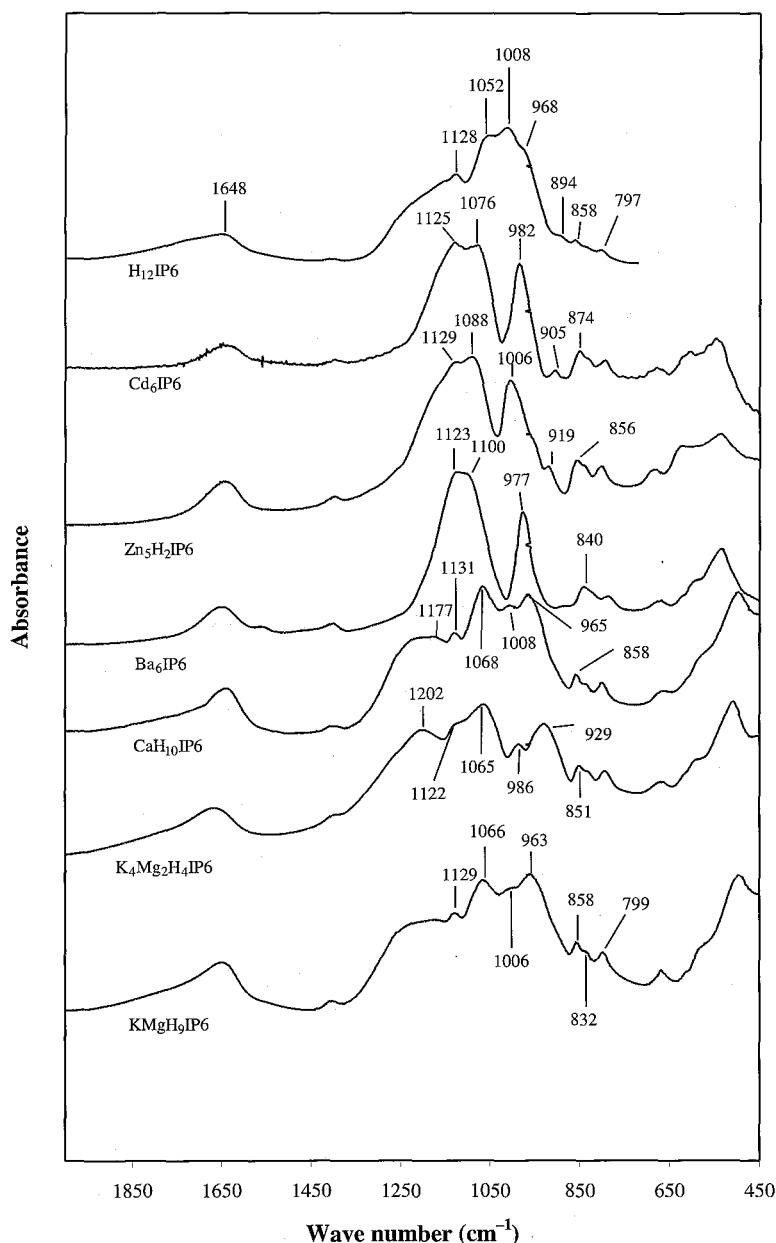


Fig. 2. Fourier transform infrared spectra of phytic acid and metal phytate (IP₆) compounds.

NMR spectra. These tubes were maintained overnight to ensure equal wetting. The solid-state ^{31}P MAS NMR spectra of these wetted samples were obtained as per the dry samples.

RESULTS

Fourier Transform Infrared Spectral Features of Metal Orthophosphate Compounds

Potassium dibasic phosphate (K_2HPO_4) showed a single strong peak at 1077 cm^{-1} and two other P-relevant bonds at 985 and 859 cm^{-1} (Fig. 1). The spectrum of sodium dibasic phosphate (Na_2HPO_4) was very similar to that of disodium phosphate. The three-peak feature of K and Na dibasic phosphates was consistent with previous reports (Chapman and Thirlwell, 1964; Kumamoto, 1965). Calcium dibasic phosphate contained three similar peaks, but an additional peak appeared at 1132 cm^{-1} (Fig. 1). The monobasic phosphates, NaH_2PO_4 and $\text{Ca}(\text{H}_2\text{PO}_4)_2$, yielded four peaks in a range from 970 to 1300 cm^{-1} ; however, these peaks were

much weaker than those of dibasic phosphates (Fig. 1). Similar to other divalent metal tribasic phosphates (Rulmont et al., 1991; He et al., 2006c), $\text{Cd}_3(\text{PO}_4)_2$ and $\text{Zn}_3(\text{PO}_4)_2$ demonstrated complicated FT-IR features. There were two broad bands at 1016 and 1068 cm^{-1} and a shoulder band at 919 cm^{-1} in the spectrum of $\text{Cd}_3(\text{PO}_4)_2$. There were five strong bands at 992 , 1002 , 1020 , 1073 , 1107 , and 1125 cm^{-1} in the spectrum of $\text{Zn}_3(\text{PO}_4)_2$.

Fourier Transform Infrared Spectral Features of Metal Phytate Compounds

Phytic acid (H_{12}IP_6) demonstrated a broad hump comprised of three peaks at 968 , 1008 , and 1052 cm^{-1} ; a peak at 1128 cm^{-1} ; and three weak peaks at 797 , 858 , and 894 cm^{-1} (Fig. 2). When the protons of the OH groups were partially substituted by metals, the strongest peak of H_{12}IP_6 at 1008 cm^{-1} was clouded by more prominent peaks on either side, as in the spectra of KMgH_9IP_6 and $\text{CaH}_{10}\text{IP}_6$, or they

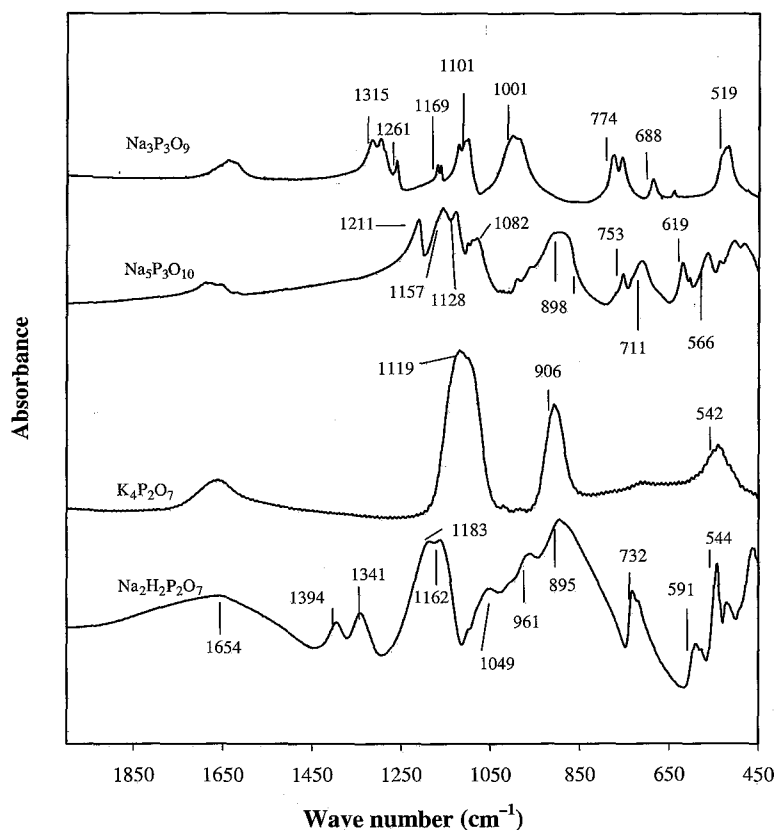


Fig. 3. Fourier transform infrared spectra of pyrophosphate and triphosphate compounds. $\text{Na}_5\text{P}_3\text{O}_{10}$ = pentasodium tripolyphosphate; $\text{Na}_3\text{P}_3\text{O}_9$ = trisodium trimetaphosphate.

disappeared, as in the spectra of Cd_6IP_6 , $\text{Zn}_5\text{H}_2\text{IP}_6$, Ba_6IP_6 , and $\text{K}_4\text{Mg}_2\text{H}_4\text{IP}_6$. Ba-, Zn-, and Cd-phytates demonstrated FT-IR spectra typical of divalent metal phytate compounds (He et al., 2006c): a sharp peak around 1000 cm^{-1} and splitting or nonsplitting peaks at higher wave numbers. Although nonsplitting broad peaks were a feature of II(A) metal phytate compounds (Ba_6IP_6 in Fig 2; and Ca_6IP_6 and Mg_6IP_6 in He et al., 2006c), splitting broad peaks were observed in divalent

transition metal phytate compounds Cd_6IP_6 and Zn_6IP_6 (Fig. 2), as well as Mn and Cu phytates (He et al., 2006c). Another feature of metal phytates different from that of phytic acid was stronger triplet peaks from 790 to 900 cm^{-1} .

Fourier Transform Infrared Spectral Features of Pyro(di)phosphates and Triphosphates

In addition to P-O bonds, these polyphosphate compounds contain bridged P-O-P bonds that limit bond movement, a characteristic

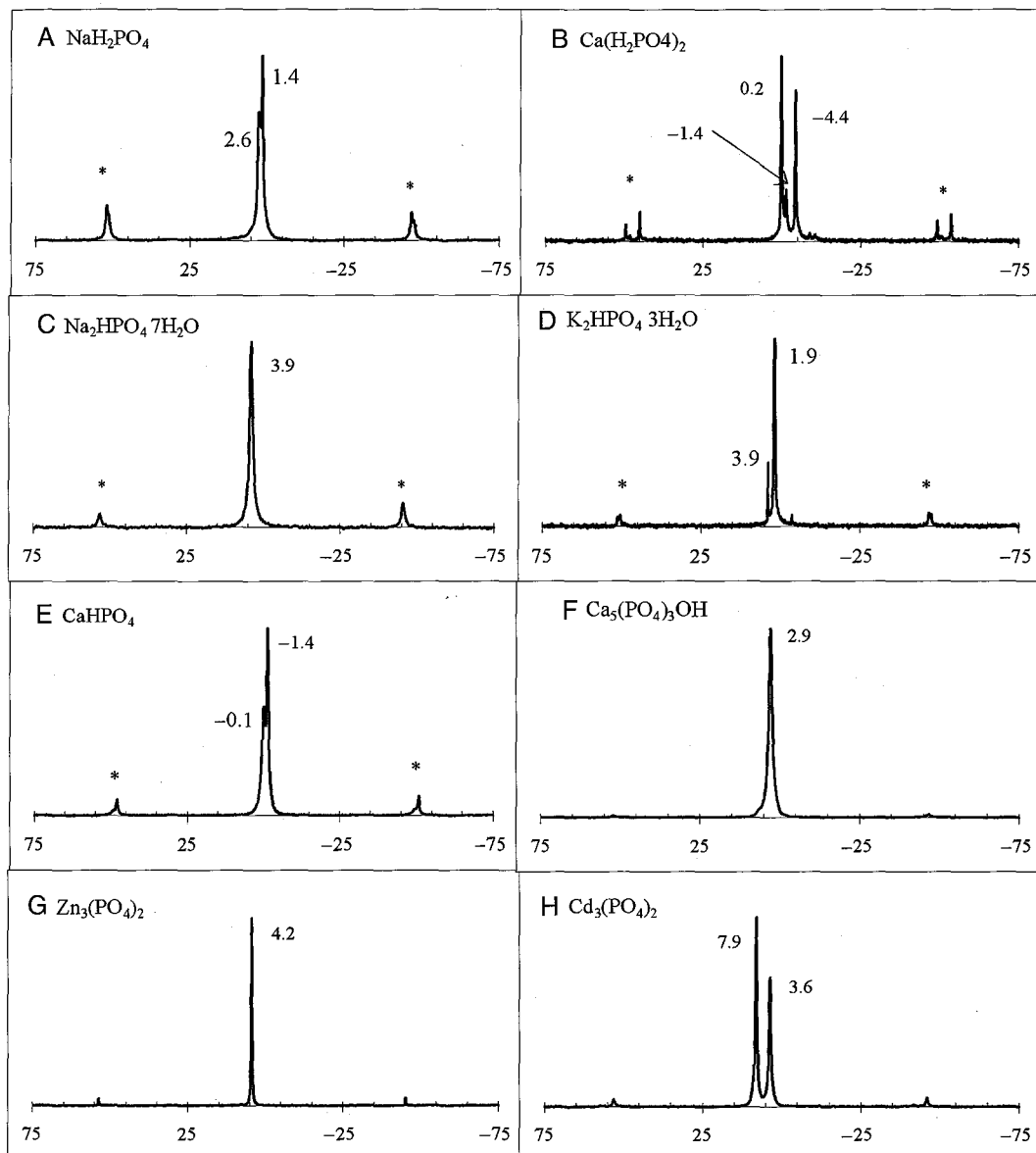


Fig. 4. Solid-state ^{31}P MAS NMR spectra of metal phosphate compounds. The chemical shift of peaks is in parts per million. Spinning sidebands are labeled with an asterisk.

shown in the spectra (Fig. 3). The spectrum of tetrapotassium pyrophosphate ($K_4P_2O_7$) was characterized by two strong peaks at 906 and 1119 cm^{-1} (Fig. 3), corresponding to the splitting of the P-O peak in orthophosphate at 1011 cm^{-1} (Rulmont et al., 1991; He et al., 2006c). However, in comparison, the spectrum of disodium dihydrogen pyrophosphate ($Na_2H_2P_2O_7$) was very complicated, with multiple peaks from 732 to 1394 cm^{-1} due to the

existence of both HO-P and free (metal) P-O bonds.

Pentasodium tripolyphosphate ($Na_5P_3O_{10}$) is a short-chain triphosphate, whereas trisodium trimetaphosphate ($Na_3P_3O_9$) is a small-ring triphosphate. The spectra of both compounds were complicated. Peaks with variable intensity were observed in the region from 1100 to 1200 cm^{-1} . As reported previously (Rulmont et al., 1991), the most characteristic feature of these

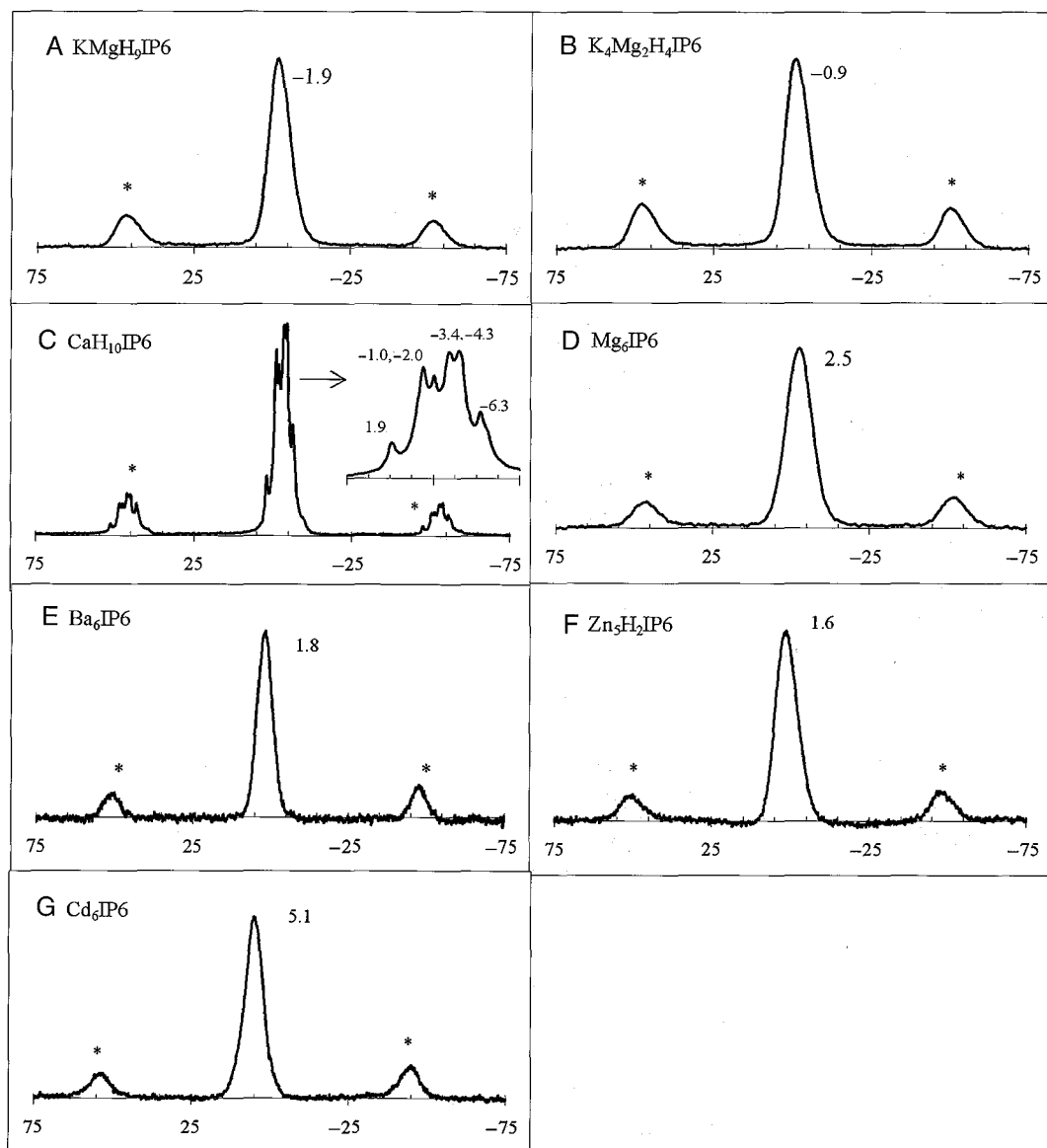


Fig. 5. Solid-state ^{31}P MAS NMR spectra of metal phytate (IP6) compounds. The chemical shift of peaks is in parts per million. Spinning sidebands are labeled with an asterisk.

polyphosphates was the existence of one or several strong peaks beyond 1200 cm^{-1} .

Solid-State ^{31}P NMR Spectral Features of Metal Orthophosphate Compounds

The effect of metal ions was obvious in spectra yielding a range of peaks at different chemical shifts among the same type of phosphate (i.e., H_2PO_4^- , HPO_4^{2-} , PO_4^{3-}) (Fig. 4). The ^{31}P MAS NMR spectrum of sodium monobasic phosphate (NaH_2PO_4) shows two main peaks at 2.6 and 1.4 ppm. But three peaks were observed in the spectrum of calcium monobasic phosphate at 0.2, -1.4, and -4.4 ppm. The spectrum of sodium dibasic phosphate hydrate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$) shows a main peak at 3.9 ppm, but the spectrum of potassium dibasic phosphate hydrate ($\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$) has a major peak at 1.9 ppm and a sharp peak at 3.9 ppm. Similarly, the spectrum of calcium dibasic phosphate (CaHPO_4) displays two isotropic peaks at -0.1 and -1.4 ppm. Although there was only one major peak in the spectra of Ca and Zn tribasic phosphates, two well-separated peaks at 7.9 and 3.6 ppm appeared in the spectrum of Cd tribasic phosphate. Multiple solid-state ^{31}P MAS NMR peaks of calcium

monobasic phosphates and dibasic phosphates have been previously reported and attributed to two crystallographically distinct phosphate groups in Ca phosphate compounds (Rothwell et al., 1980). Half of the phosphates in CaHPO_4 are bonded to one hydrogen in HPO_4^{2-} , whereas the other half are characterized by one proton in a symmetrically bridging hydrogen bond and one proton statistically distributed between two centrosymmetric positions. Similarly, one type of $\text{Ca}(\text{H}_2\text{PO}_4)_2$ consists of dimeric H_2PO_4^- groups; the other type is a hydrogen-bonded chain of phosphates. Multiple crystallographically distinct phosphate groups could be used to explain the multiple chemical shift peaks observed in our samples. In addition to different ^{31}P NMR major chemical shifts in the seven phosphate compounds, an interesting observation was the decreasing intensity of the spinning sidebands in these spectra in the order of monobasic > dibasic > tribasic phosphates.

Solid-State ^{31}P MAS NMR Spectral Features of Metal Phytate Compounds

As observed for orthophosphates, the spectra of phytate compounds were affected by the metals (Fig. 5). Even for phytate compounds

TABLE 2
Effect of wetting on solid-state ^{31}P MAS NMR spectral features of phytate (IP6) compounds

Phytate	Chemical shift				Spin sideband			
	Dry sample		Wet sample		Dry sample		Wet sample	
	Position ppm	Height %	Position ppm	Height %	Position ppm	Height %	Position ppm	Height %
$\text{Na}_{12}\text{IP}_6^\dagger$	7.5	110.0	4.5	68.2	56.7	16.4	53.9	9.9
			6.6	100.0	-41.7	25.7	-44.7	14.8
$\text{K}_2\text{H}_{10}\text{IP}_6$	-0.7	110.0	-0.1	116.7	48.6	16.4	49.2	1.4
			-0.8	82.2	-49.8	14.0	46.9	1.4
			-1.3	47.0			-49.3	1.6
			-2.6	7.0				
KMgH_9IP_6	-1.9	110.0	-0.9	116.7	46.8	18.9	ND [‡]	—
			-1.8	95.0	-51.4	15.6		
$\text{K}_4\text{Mg}_2\text{H}_4\text{IP}_6$	-0.9	110.0	0.0	111.8	48.1	26.0	-52.0	0.8
			-0.4	116.7	-49.7	23.5		
			-0.9	61.5				
Mg_6IP_6	-2.5	110.0	-0.4	100.0	45.9	16.0	48.2	14.1
					-51.8	18.7	-49.4	15.5
Ca_6IP_6	0.5	110.0	0.9	83.7	49.8	14.4	49.1	10.9
					-48.8	21.2	-49.0	15.5
Ba_6IP_6	1.8	100.0	1.8	95.0	49.9	13.2	50.7	14.9
					-47.7	17.6	48.0	15.6
Al_4IP_6	-14.8	139.5	-13.8	100.0	36.6	19.0	37.0	13.6
					-63.0	17.3	-61.8	13.2

[†]Data of dry samples of $\text{Na}_{12}\text{IP}_6$, $\text{K}_2\text{H}_{10}\text{IP}_6$, Ca_6IP_6 , and Al_4IP_6 were derived from (He et al., 2007).

[‡]Not detected.

with the same metal moieties (e.g., KMgH_9IP_6 ; $\text{K}_4\text{Mg}_2\text{H}_4\text{IP}_6$), the chemical shift of peaks differed, presumably because of the number of metal-O-P or H-O-P bonds in each phytate compound. Similarly, the major peak of $\text{CaH}_{10}\text{IP}_6$ centered at -3.4 ppm was different from that of Ca_6IP_6 at 0.5 ppm as found by He et al. (2007). It is noticeable that unlike other spectra, the major peak of $\text{CaH}_{10}\text{IP}_6$ was split to six distinguishable sub peaks (Fig. 5C). In addition to individual spectral characteristics, there was a general feature that distinguished phytate compounds from orthophosphate compounds—both the main peaks and spinning sidebands were much broader than those of orthophosphate (cf. Figs. 4 and 5).

The spectra of eight wetted phytate samples were obtained. Compared with dry samples, more than one chemical shift peak appeared in the spectra of alkaline metal (Na and K) phytates and K/Mg phytates (Table 2), indicating a transition to other P compounds. Meanwhile, the height of spinning sidebands for metal (Na and K) phytates and K/Mg phytates was shorter or had disappeared. There was still one peak in wetted metal(II) and metal(III) phytate compounds, but the chemical shift changed in three of the four compounds. The spinning sidebands were unchanged in the wetted metal(II) and metal(III) phytate compounds, compared with their dry counterparts. From these observations, we concluded that wetting affected the spectral characteristics of phytate and other P compounds. This effect was greater in soluble compounds.

Solid-State ^{31}P NMR Spectral Features of Pyro(di)phosphates and Triphosphates

With sharp peaks observed, spectra of the four polyphosphate compounds were more like those of orthophosphates than phytate compounds (Fig. 6). However, the chemical shifts were generally located upfield of those for orthophosphate compounds. The intensity of the spinning sidebands in these polyphosphates was stronger than those of orthophosphates, but weaker than those of phytate compounds. Apart from $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$, the other three compounds ($\text{K}_4\text{P}_2\text{O}_7$, $\text{Na}_5\text{P}_3\text{O}_{10}$, and $\text{Na}_3\text{P}_3\text{O}_9$) demonstrated asymmetric spinning sidebands. The right spinning sidebands were more intense than the downfield side of the spectra of $\text{K}_4\text{P}_2\text{O}_7$ and $\text{Na}_5\text{P}_3\text{O}_{10}$. In contrast, the upfield spinning

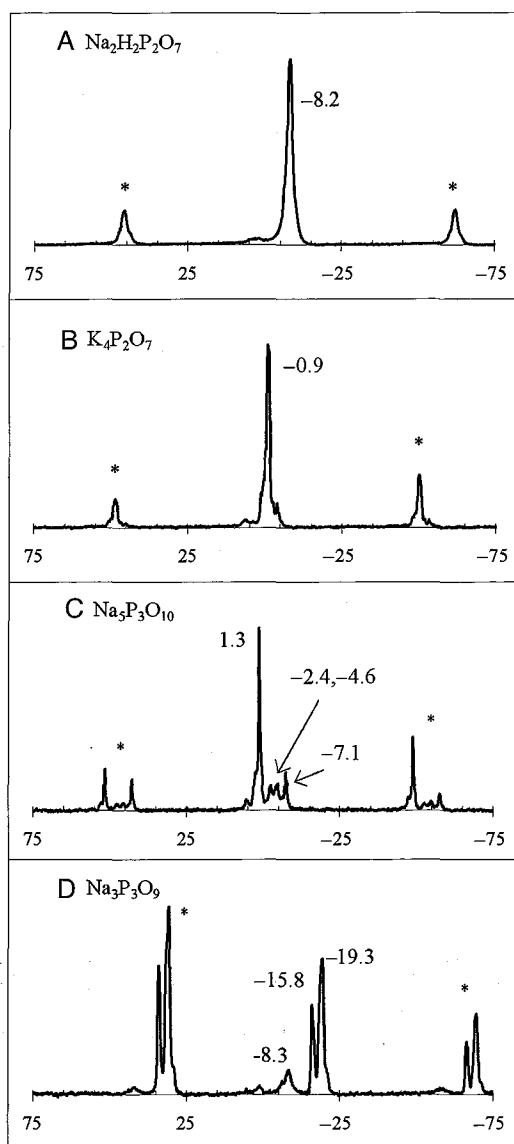


Fig. 6. Solid-state ^{31}P MAS NMR spectra of pyrophosphate and triphosphate compounds. The chemical shift of peaks is in parts per million. Spinning sidebands are labeled with an asterisk.

sidebands of $\text{Na}_3\text{P}_3\text{O}_9$ were stronger, even higher than the isotropic peaks.

DISCUSSION

Infrared spectroscopy has been used to identify soil constituents and study structural features of both inorganic and organic components (Francioso et al., 1996; Johnston and

Aochi, 1996; Celi et al., 1999; Ding et al., 2002). The orthophosphate ion (PO_4^{3-}) is a symmetrical tetragonal pentaatomic molecule that shows a single degenerate vibration peak at about 1010 cm^{-1} in IR spectra (Chapman and Thirlwell, 1964; Kumamoto, 1965; He et al., 2006c). Any external distortion effects on the PO_4^{3-} orthotetragonal structure would cause the P-O bonds in PO_4^{3-} to vibrate and bend asymmetrically, producing more characteristic IR bands (Chapman and Thirlwell, 1964; Kumamoto, 1965; Rulmont et al., 1991). Therefore, this technique has been used to investigate inorganic phosphate adsorption on soil mineral components (Atkinson et al., 1974; Persson et al., 1996; Tejedor-Tejedor and Anderson, 1990; Arai and Sparks, 2001) and the interaction of phytate on clays (Celi et al., 1999). These reports provide limited information on the effects of metal ions on IR spectra of phosphate compounds.

In addition to previous data (He et al., 2006c), this work provided more FT-IR spectra of metal phytate compounds. These spectra (Fig. 2 and those in He et al., 2006c) clearly indicate that the major FT-IR bands in the $900\text{--}1150\text{ cm}^{-1}$ range shifted toward higher wave numbers with the increase of metal valents. These shifts were apparently because of the different electronegativity of these metal ions. Generally, the lower the electronegativity, the stronger the covalent character of a metal bond. As a result, the corresponding P-O bond is weakened, leading to the lower vibration frequency (higher wave numbers) (Rulmont et al., 1991). Thus, the characteristic band positions in the $900\text{--}1150\text{ cm}^{-1}$ range could be used in investigating competitive binding of phytate with different metal ions.

Comparative analysis of FT-IR spectra of metal orthophosphates and phytates demonstrated distinct FT-IR differences among the compounds. In general, spectra became more complicated with the change in phosphate structure from an orthotetragon (PO_4^{3-}) to distorted tetragons (XPO_4^{2-} , X_2PO_4^- , or X_3PO_4 , X = H, C, or M). The spectra of $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ and $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ had three-peak features and that of CaHPO_4 showed an additional peak at 1132 cm^{-1} (Fig. 1) due to bending of two adjacent P-O bonds by the divalent property of Ca(II). Phytic acid, which contains six H_2CPO_4 units, had a spectrum (Fig. 2) that was more like a hybrid spectrum of Na or K_2HPO_4 and CaHPO_4 than of NaH_2PO_4

or $\text{Ca}(\text{H}_2\text{PO}_4)_2$ (Fig. 2). This observation implied that C-O-P bonds in phytic acid (H_{12}IP_6) were restrained like H-O-P bonds in dibasic phosphate compounds, and H-O-P bond in phytic acid vibrated like ionic M-O-P in dibasic compounds. In the spectra of solid or aqueous HPO_4^{2-} ions, the peak around 850 cm^{-1} is assigned to the P-O-(H) stretch vibration (Chapman and Thirlwell, 1964). We can, therefore, assume that peaks from 790 to 900 cm^{-1} in the spectrum of phytic acid were contributed by the P-O-(C) stretch vibration. A hybrid spectral feature and triplet peaks in the $790\text{--}900\text{ cm}^{-1}$ range were caused by the existence of three different types of H_2CPO_4 moieties in each phytic acid molecule (Turner et al., 2002b; He et al., 2006c). With metal phytates, the triplet-peak feature in this range became more evident although it looked more like a doublet in some spectra. Although there was only one or no peak in the $790\text{--}900\text{ cm}^{-1}$ range of the spectra of other phosphate compounds in Fig. 1 and 3, the unique FT-IR multiple peaks in the $790\text{--}900\text{ cm}^{-1}$ range could be used to distinguish phytates from other P compounds (He et al., 2006c). In addition, this work also demonstrated that the spectral features of partially deprotonated metal phytates (i.e., KMgH_9IP_6 , $\text{K}_4\text{Mg}_2\text{H}_4\text{IP}_6$, and $\text{CaH}_{10}\text{IP}_6$), which kept some spectral bands of phytic acid, could be useful for studying the mechanism of association and dissociation of metal phytates.

As it directly detects the stretching and bending vibration of chemical bonds, FT-IR analysis is a powerful tool to study fundamental soil P chemistry. This work provides a basis for the study of P chemistry, especially metal phytates, using FT-IR. Current FT-IR information is very limited (Celi et al., 1999; Guan et al., 2006). Furthermore, this work would be useful in the study of phosphate adsorption on soil mineral components (i.e., metal oxides or hydroxides) because the mechanism of distortion of the PO_4^{3-} orthotetragon in solids or aqueous solutions is the same (Chapman and Thirlwell, 1964; Tejedor-Tejedor and Anderson, 1990; Persson et al., 1996; Arai and Sparks, 2001).

Solid-state ^{31}P NMR is another spectroscopic technique used in the study of P in the environment. Unlike FT-IR, which is applicable only to pure chemicals or at least partially purified soil components, solid-state ^{31}P MAS NMR can analyze the P species in undisturbed real-world samples. To do this, the solid-state

^{31}P MAS NMR spectra of samples are usually analyzed, and the peaks in the resulting spectra deconvoluted and assigned using a series of reference spectra of model P compounds (Shand et al., 1999; McDowell et al., 2002; McDowell et al., 2003; Hunger et al., 2004; Jayasundera et al., 2005). Although these studies have shed light on the metal speciation of inorganic P, the effect of counter metal ions on the spectral feature of phytates is often overlooked (Shand et al., 1999; Hunger et al., 2004; Jayasundera et al., 2005). He et al. (2007) provided the peak assignments for four metal phytates for solid-state ^{31}P MAS NMR spectra, and more assignments for phytate compounds and inorganic phosphates were obtained in this work. This enables us to better identify phytates and track changes in P speciation in environmental samples with solid-state ^{31}P MAS NMR analysis.

In addition to the specific solid-state ^{31}P MAS NMR peak assignments for each P compound, we can derive three general conclusions based on the different spectral features in Figs. 4–6 and Table 2. The first is that peaks for phytate compounds were broad, whereas those of inorganic P compounds were much narrower. The presence of paramagnetic Fe and Mn has often been thought to be responsible for broadening NMR peaks (Shand et al., 1999; Cade-Menun, 2005). However, Shand et al. (1999) reported that decreasing Fe and Mn concentrations in their soil and humic acid samples produced little change in solid-state ^{31}P MAS NMR resolution. They concluded that peak broadening in solid-state ^{31}P MAS NMR spectra of soil-humic substances does not stem from paramagnetic substances, but from the variable way that P species are associated with the organic phase. This work supports their conclusion. We also observed broad peaks in the spectra of sodium glucose-6-phosphate, β -NAD, and β -NADP, but not in the spectrum of sodium glycerolphosphate (data not shown). This suggests that a structurally complex organic moiety contributes to the broad peak. The peak feature of $\text{CaH}_{10}\text{IP}_6$ (Fig. 5) warrants further discussion. This chemical (5 g) was purchased from Sigma-Aldrich. It arrived as a white powder but coagulated upon exposure to air and presumably moisture. We reground it before analysis. However, because of a lack of supply, we were not able to confirm whether the chemical shift of $\text{CaH}_{10}\text{IP}_6$ was because of coagulation. Additional pretreatment by drying may result in better-resolved spectra, because

others (e.g., Sakellariou et al., 2003) have shown that broad solid-state ^{31}P MAS NMR peaks are comprised of numerous finer peaks.

The second general conclusion concerns spinning sidebands. Although they are not presented or fully discussed in some literature (Hunger et al., 2004; Jayasundera et al., 2005), spinning sidebands have been used to characterize solid calcium phosphate phases. For example, apatite has less intense spinning sidebands compared with protonated Ca-P at slow MAS speeds (1–4 kHz) (Rothwell et al., 1980). Frossard et al. (1994) found that spinning sidebands were not modified in the spectra of an urban sewage sludge after sequential extraction with H_2O , NaHCO_3 , and NaOH , but they almost disappeared after subsequent HCl extraction. McBeath et al. (2006) concluded that Ca orthophosphate and Ca pyrophosphate, which were the dominant species present in their soil samples, can be distinguished on the basis of the chemical shift of the main peaks and spinning sideband patterns. He et al. (2007) proposed that spinning sidebands may be used to distinguish phytate species from their corresponding inorganic phosphate species, especially when their major peaks are similar, such as the case between dodecasodium phytate and trisodium phosphate and between tetraaluminum phytate and aluminum phosphate. The present work provided further evidence that spinning sideband characteristics were related to the existence of P–O–(H) or P–O–C bonding in P compounds. In general, the intensity of spinning sidebands was increased in an order from phytates > protonated phosphate > deprotonated phosphates (negligible or nonexistent). The asymmetrical occurrence of spinning sidebands is helpful in identifying polyphosphates. Recently, Toor et al. (2006) also reported that spinning sidebands could be used for diagnostic purposes based on the fact that their occurrence and intensity depend on the symmetry of the chemical environment of the observed nucleus and its perturbation by chemical bonds (Bleam et al., 1989). It seems meaningful to pay the same attention to spinning sidebands as to the major chemical shifts of spectra.

Finally, keeping samples fully dry appears necessary for obtaining repeatable and high quality solid-state ^{31}P MAS NMR spectra. Although it is reported that retaining some water in samples is allowable (Hunger et al., 2004; Toor et al., 2006), our observation (Table 2) indicated that wetting samples changes both

intensity and position of the main peaks and spinning sidebands. The mechanism for this is probably through the loosening of the O-H or O-metal bond in soluble P compounds, thus changing localized magnetic environments around P-O-H or P-O-M. It is hard to compare solid-state ^{31}P MAS NMR spectral data of moist samples especially if the water content between samples is different. Therefore, we recommend use of dry samples, and we believe freeze-drying, which minimized changes in air or oven drying, would lead to the most repeatable results (McDowell, 2003).

CONCLUSIONS

Solid-state spectroscopic techniques have provided knowledge on metal speciation of inorganic P in animal manure and soil. However, it has not been successful in resolving these spectral data in detail on specific metal organic P compounds due to the lack of appropriate reference spectra. In this work, we characterized the solid-state FT-IR and ^{31}P NMR spectral features of seven metal phytates (as model organic P compounds) and their inorganic P counterparts. Differences were observed in the intensity, position, and shape of the spectral peaks among these phytates and between metal phytates and their corresponding inorganic P compounds. Thus, reference spectra of these model compounds established in this work and our previous work (He et al., 2006c, 2007) would be helpful in both basic studies of metal-P interaction mechanisms and applied studies of metal speciation of organic P compounds in animal manure and soil as well as their transformation under various environmental conditions.

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